NON-INVASIVE MEASUREMENT OF LOWER RESPIRATORY CYTOKINE BIOMARKERS WITH PEXA AND ULTRASENSITIVE IMMUNOASSAYS

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PURPOSE

Detecting Proteins and Cytokines in Exhaled Air

Asthma, chronic obstructive pulmonary disease (COPD), idiopathic pulmonary fibrosis (IPF), lung transplant rejection, and even SARS-CoV-2 infection, is characterized by inflammation mediated by cytokines and dysfunction of small airways. However, no standardized non-invasive biomarkers are available to assess small airway inflammation.

Previous non-invasive respiratory tract collections such as exhaled breath condensate are diluted with water and contaminated with oral cavity proteins. **Particles in exhaled air (PExA)** from respiratory tract lining fluid of small airways consists of lipids and proteins making them an ideal non-invasive biomarker candidate.

However, methods have not been created to measure cytokines in PExA due to their expected ultra-low abundance. We aimed to measure cytokines in PExA by combining microsampling techniques typically used for blood with ultrasensitive immunoassays.

METHOD(S)

PExA Collection and Exhalation Normalization

- We collected PExA using a specialized instrument in 3 healthy volunteers. A constant 100 L of breath was collected and corrected for acquired particle mass. The PExA were collected onto a 25 mm Millipore membrane.
- The particles expired were normalized between individuals by in-line measurement of collected particle mass.

PExA Protein Extraction

- The membrane was punched into 10 identical 1.2 mm plugs to attain 25 ng of PExA for each sample and placed into a polypropylene tube.
- Each plug was extracted into 25 ul of buffer. Several extraction buffers and temperatures were compared including buffers commonly used for dry blood spots (DBS), and final samples were extracted using phosphate-buffered saline (PBS) containing 1% bovine serum albumin, 0.05% Tween-20, and protease inhibitor.

Biomarker Analysis

- We measured IgG using MesoScale Discovery and biomarker kits following manufacturer protocols.
- We measured IL-6, TNF-a, and IL-10 in extracted samples using and Quanterix Simoa and Somru Bioscience Amplatto immuno-PCR biomarker kits following manufacturer protocols.

RESULT(S)

Buffer Optimization and Protein Measurement

- IgG was detected in all PExA samples and IL-10 and TNF-a were detectable in 25% of PExA samples.
- IL-6 was below the limit of detection of the Simoa assay but was detectable with immuno-PCR.
- PBS containing buffer outperformed acetonitrile or ammonia containing DBS extraction buffers.
- Further studies are underway to optimize intra-sample variability and determine correlation of PExA cytokines with respiratory diseases.

CONCLUSION(S) AND FUTURE DIRECTIONS

PExA collection and analysis with ultrasensitive immunoassays is a valid non-invasive method to monitor cytokines sampled from the lower respiratory tract.

This method avoids the pitfalls of exhaled breath condensate (EBC) collection because exhaled particles may be missed by the condenser without being collected. In addition, this method also avoids the problem of contamination from the oral cavity, because no particles can be formed from a liquid surface unless the flow is extremely high. Consistent with this finding, amylase (a saliva marker) is not found in PExA.

This method may be useful for biomarker measurement in asthma, chronic obstructive pulmonary disease (COPD), idiopathic pulmonary fibrosis (IPF), tobacco injury, lung transplant rejection, and even SARS-CoV-2 infection.

ACKNOWLEDGEMENTS

We thank SomruBioscience for assistance with immuno-PCR experimentation. We thank Jörgen Östling, PhD at PExA AB for providing the samples for biomarker analysis and scientific expertise.

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Novel Methods to **Measure Protein and Cytokine Biomarkers Non-Invasively** in Lower Respiratory Tract Lining Fluid for **Respiratory Diseases** Using Particles in Exhaled Air (PExA)

WHAT IS PEXA?

Non-Invasive Lower Respiratory Biomarker for COPD, Asthma, & Viral Infections

Particles in exhaled air (PExA) collects droplets are formed and collected during expiration as an alternative to invasive bronchoalveolar lavage. Proteins, inflammatory markers, and lipids are deposited onto a filter substrate.

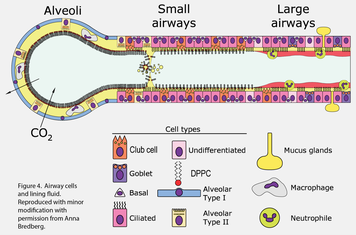
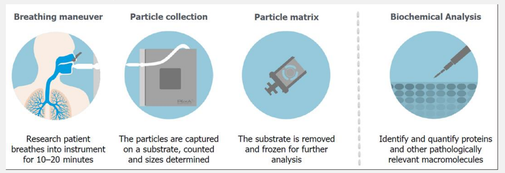
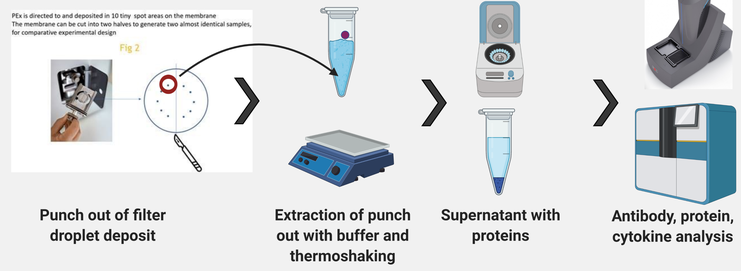


Figure 4. Airway cells and lining fluid. Reproduced with minor modification with permission from Anna Bredberg.



NOVEL PROTEIN ISOLATION METHOD

Because proteins are expected to have low abundance in PExA, we developed a filter punch out and protein extraction process that improves recovery of low abundance proteins in exhaled air. Different extraction buffers such as dry blood spot buffers were compared.



Punch out of filter droplet deposit

Extraction of punch out with buffer and thermoshaking

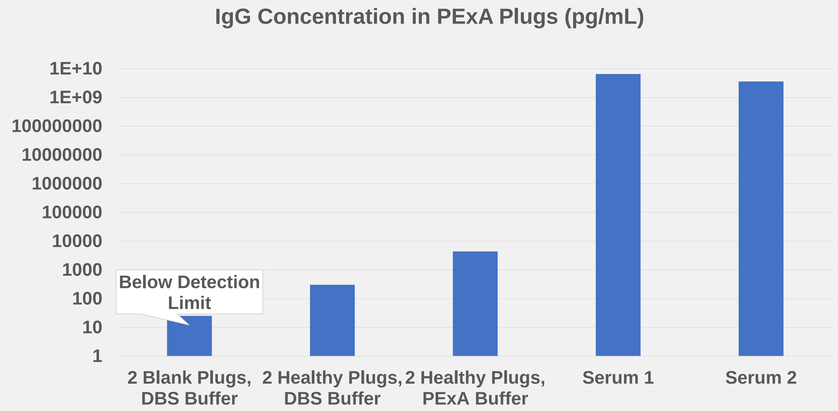
Supernatant with proteins

Antibody, protein, cytokine analysis

ANTIBODY MEASUREMENT

MSD ECL Detection of Human IgG in PExA

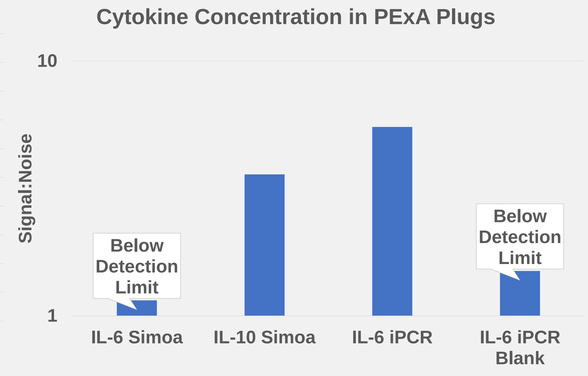
Human IgG can be detected in expired air particles using the punch out process indicating other proteins and inflammatory markers can be detected.



ULTRASENSITIVE CYTOKINE MEASUREMENT

Quanterix Simoa and Amplatto iPCR in PExA

We were able to detect IL-10 using Quanterix Simoa and IL-6 using immunoPCR in healthy volunteer PExA.



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